i An update to this article is included at the end

foundation for modern studies of how and why sexual dimorphisms arose and evolved; with the advent of new technologies the near future will certainly provide deeper insights into the molecular basis of these processes.

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Screening mammal biodiversity using DNA from leeches

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With nearly one quarter of mammalian species threatened, an accurate description of their distribution and conservation status is needed [1]. For rare, shy or cryptic species, existing monitoring methods are often prohibitively expensive or unreliable. The problem is particularly acute in tropical forests, where a disproportionate number of species are listed by IUCN as 'data deficient' [2], due to the difficulty of monitoring with conventional approaches. This presents serious obstacles to conservation management. We, here, describe a new screening tool, the analysis of mammalian DNA extracted from haematophagous leeches. By demonstrating that PCR amplifiable mammalian blood DNA survives for at least four months post feeding in haematophagous *Hirudo* spp. leeches, we hypothesise that most wild caught adult leeches will contain DNA traces of their last blood meal. We subsequently demonstrate the efficacy of the method, by testing it *in situ* using terrestrial *Haemadipsa* spp. leeches caught in a tropical Vietnamese rainforest setting, and identify cryptic, rare and newly discovered mammalian species. We propose that DNA from leeches represents a quick, cost-effective and standardised way to obtain basic data on mammalian biodiversity and species occupancy, facilitating efficient use of limited conservation resources.

An emerging tool for assessing mammalian biodiversity is the profiling of DNA extracted from micropredators. In addition to ticks and mosquitoes [2], haematophagous leeches represent promising candidates as, following feeding, they store concentrated blood for several months [3]. Furthermore, several studies have demonstrated that in the medical leech (*Hirudo medicinalis*) viruses remain detectable in the blood meal for up to 27 weeks, indicating viral nucleic acid survival [4,5]. To examine whether PCR amplifiable mammalian DNA persists in ingested blood, we fed 26 medical leeches (*Hirudo* spp.) freshly drawn goat (*Capra hircus*) blood (Supplemental information) then sequentially killed them over 141 days. Following extraction of total DNA, a goat-specific quantitative PCR assay demonstrated mitochondrial DNA (mtDNA) survival in all leeches, thus persistence of goat DNA, for at least 4 months (Figure 1A; Supplemental information).

We subsequently applied the method to monitor terrestrial mammal biodiversity in a challenging environment. *Haemadipsa* spp. leeches were collected in a densely forested biotope in the Central Annamite region of Vietnam (Figure 1B; Supplemental information), in which five new mammal species have recently been discovered. Despite the interest these discoveries have generated, including adoption of the saola (*Pseudoryx nghetinhensis*) as a regional flagship species by the World Wildlife Fund, all attempts to develop standardised survey and monitoring techniques for these species have failed.

Remarkably, 21 out of 25 leeches tested yielded mammalian mtDNA sequences, representing six species spanning three orders — Artiodactyla, Carnivora and Lagomorpha (Figure 1B; Supplemental information). We deliberately chose PCR assays that are unable to PCR amplify human DNA in order to prevent false positives derived from human contamination of the leeches at time of sampling (Supplemental information). Therefore, it is possible that human DNA is present in the four samples that failed to yield an amplicon; thus, the value is a conservative estimate of the blood-meal-derived mammalian DNA prevalence in the leeches. Although multiple clones were sequenced per amplicon, no leech yielded sequences from more than one species, suggesting that rapid decline in blood meal DNA concentration over time (Supplemental information) will render DNA levels derived from a new feeding to be greatly higher than those from previous feedings. Two of the detected species have been recently described, the Truong Son muntjac (*Muntiacus truongsonensis,* one leech) and

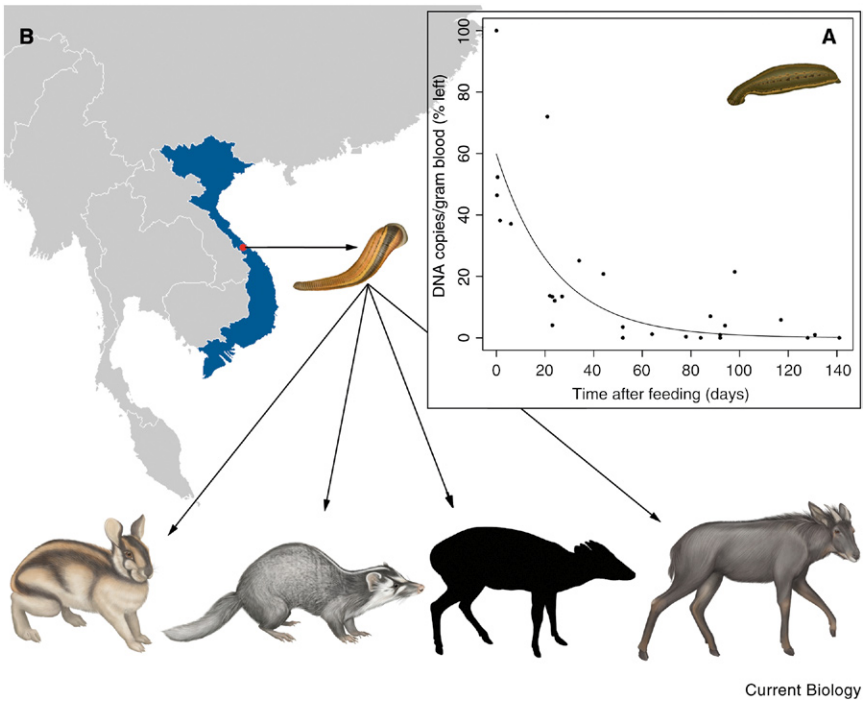

Figure 1. Monitoring mammals with leeches.
(A) Survival of mtDNA in goat blood ingested by *Hirudo medicinalis* over time, relative to freshly drawn sample (100%, ca. 2.4E+09 mtDNA copies/gram blood). Mitochondrial DNA remained detectable in all fed leeches, with a minimum observed level at 1.6E+04 mtDNA/gram blood ingested. The line shows a simple exponential decay model, $p < 0.001$, $R^2 = 0.43$ (Supplemental information). (B) Vietnamese field site location and examples of mammals identified in *Haemadipsa* spp. leeches. From left to right: Annamite striped rabbit, small-toothed ferret-badger, Truong Son munjtac (coat coloration and markings remain unknown), serow. Pictures do not reflect true size proportions. See also Supplemental information.

Annamite striped rabbit (*Nesolagus timminsi,* four leeches). Although IUCN lists both as 'data deficient', future data are expected to confirm their threatened status. Six leeches contained small-toothed ferret-badger (*Melogale moschata*) DNA, a cryptic species impossible to discriminate from related *M. personata* without handling. Three contained serow (*Capricornis maritimus*) DNA, a near-threatened species in decline. Lastly cow (*Bos taurus*) and pig (*Sus scrofa*) DNA were observed in two and five leeches, respectively. Pigs are the most common large terrestrial mammal in the area and cows are used to transport timber.

Terrestrial *Haemadipsidae* leeches are ideal for this method, due to their diverse prey base and readiness to attack humans, making them easy to collect. Haemadipsids abound in the humid forests of Southeast Asia, which have one of the most poorly described and most threatened mammal faunas in the world [1], and are present in Madagascar, South Asia and Australia. Elsewhere, as the *Hirudo* data show, aquatic leeches may prove useful. Aside from leech dietary revelations, the method has potential to revolutionise mammal detection surveys in tropical habitats. Throughout tropical Asia, mammal populations are severely depleted by hunting, remaining individuals are wary of humans, and habitats are frequently dense, rugged and humid, making detection of many species challenging. For example, although *N. timminsi* has been suspected in the survey area since its description in 1996, and over 2000 nights of camera trapping have been performed, this is the first confirmed record. Also, although *Melogale* have been detected using camera traps, the two species cannot be distinguished by sight; thus, our result is the first confirmed *M. moschata* for Bach Ma National Park. Similarly, the *M. truongsonensis* observation is the first confirmed record from the Saola Nature Reserve.

The potential for leeches in mammal surveys goes beyond making simple *ad hoc* records. Datasets of presence records can be used for inductive modelling of geographic range or for estimating total occupancy across a landscape [6,7], information central to conservation. Unlike camera trapping and dung-searches, leech data collection is simple, inexpensive and can be conducted by untrained personnel. Furthermore, the laboratory methods can easily be undertaken using simple approaches. Much work is required to fully explore the potential of biodiversity monitoring of mammals (and other vertebrates), including a better understanding of leech feeding and dispersal behavior. However, our results demonstrate that profiling DNA extracted from haematophagous terrestrial leeches is a quick, cost-effective, and standardised way to obtain basic data on the species occupancy of known mammals.

Supplemental Information

Supplemental Information includes experimental procedures and two tables and can be found with this article online at doi:10.1016/j.cub.2012.02.058.

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Errata

Maternal Control of Nutrient Allocation in Plant Seeds by Genomic Imprinting

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(Current Biology *22*, 160–165; January 24, 2012)
Due to an author oversight, Table S2 in the online Supplemental Information for this article was incorrectly titled "Dosage Effects of Imprinted *pBet9>$_{syn}$Meg1*." The correct title for Table S2 should be "Dosage Effects of Nonimprinted *pBet9>$_{syn}$Meg1*." The authors apologize for any confusion this error may have caused.

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Screening mammal biodiversity using DNA from leeches

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(Current Biology *22*, R262–R263; April 24, 2012)
Due to an author oversight, the online Supplemental Information for this Correspondence incorrectly states the geographic proximity (30 km) of the source of the most similar DNA sequence in GenBank to the muntjac DNA sequences we recovered from the leeches. The correct proximity is within 55 km. This error does not affect the results of our study. The authors apologize for any inconvenience this error may have caused.

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